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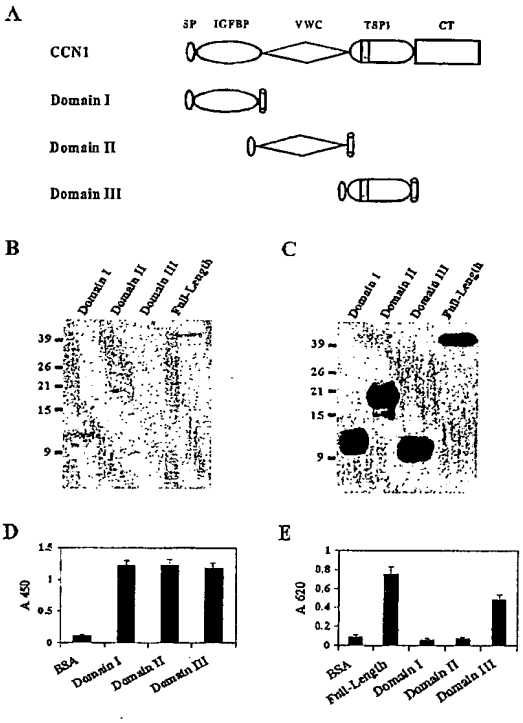
(54) Title: CCN1 COMPOSITIONS AND METHODS



(57) Abstract: The angiogenic inducer CCN1 (cysteine-rich 61, CYR61), a secreted matricellular protein of the CCN family, is a ligand of multiple integrins including $\alpha_6\beta_1$. Previous studies have shown that CCN1 interaction with integrin $\alpha_6\beta_1$ mediates adhesion of fibroblasts, endothelial cells, and smooth muscle cells, as well as migration of smooth muscle cells. Recently, we have reported that CCN1-induced tubule formation of unactivated endothelial cells is also mediated through integrin $\alpha_6\beta_1$. In this study, we demonstrate that human skin fibroblasts adhere specifically to the T1 sequence (GQKCIVQTTSWSQCSKS) within domain III of CCN1, and this process is blocked by anti-a_6 and anti-b_1 monoclonal antibodies. Alanine substitution mutagenesis of the T1 sequence further defines the sequence TTSWSQCSKS as the critical determinant for mediating $\alpha_6\beta_1$-dependent adhesion. Soluble T1 peptide specifically inhibits fibroblast adhesion to CCN1 in a dose-dependent manner. Furthermore, T1 also inhibits cell adhesion to other $\alpha_6\beta_1$ ligands including CCN2 (CTGF), CCN3 (NOV), and laminin, but not to ligands of other integrins. In addition, T1 specifically inhibits $\alpha_6\beta_1$-dependent tubule formation of unactivated endothelial cells in a CCN1-containing collagen gel matrix. To confirm that T1 binds integrin $\alpha_6\beta_1$ directly, we perform affinity chromatography and show that integrin $\alpha_6\beta_1$ is isolated from an octylglucoside extract of fibroblasts on T1-coupled Affi-gel. Taken together, these findings define the T1 sequence in CCN1 as a novel binding motif for integrin $\alpha_6\beta_1$, and form the basis for the development of peptide mimetics to examine the functional role of $\alpha_6\beta_1$ in angiogenesis.

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